1: Genome Res 1999 May;9(5):463-70 Related Articles, Books, LinkO

Automated filtration-based high-throughput plasmid preparation system.

Itoh M, Kitsunai T, Akiyama J, Shibata K, Izawa M, Kawai J, Tomaru Y, Carninci P, Shibata Y, Ozawa Y, Muramatsu M, Okazaki Y, Hayashizaki Y.

Laboratory for Genome Exploration Research Group, Genomic Sciences Center (GSC) and Genome Science Laboratory, Tsukuba Life Science Cente The Institute of Physical and Chemical Research (RIKEN), Core Research o Evolutional Science and Technology, JapanA

Current methods of plasmid preparation do not allow for large capacity automated processing. We have developed an automated high-throughput system that prepares plasmid DNA for large-scale sequencing. This system i based on our previously reported filtration method. In this method, cell harvesting, alkaline lysis, and plasmid purification occur in a single 96-well microtiter plate from which sequence-ready DNA samples are collected. The plates are designed to allow all reagents to be injected from above the wells and the spent reagents to be aspirated from below. This design has enabled u to build a linear process plasmid preparation system consisting of an automated filter plate stacker and a 21-stage automated plasmid preparator. The 96-well plates used are outfitted with glass-filters that trap Escherichia coli before the plates are stacked in the automated stacker. The plates move from the stacker to each of the 21 stages of the preparator. At specific stages, various reagents or chemicals are injected into the wells from above. Finally, the plates are collected in the second stacker. The optimal throughput of the preparator is 40,000 samples in 17.5 hr. Here, we describe a pilot experiment preparing 15,360 templates in 160 specially designed 96-well glass-filter plates. The prepared plasmids were subjected to restriction digestion, DNA sequencing, and transcriptional sequencing.

PMID: 10330126 [PubMed - indexed for MEDLINE]

1: Nucleic Acids Res 1998 Oct 1;26(19):4524-8 Related Articles, Books, LinkO

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Purification of plasmids by triplex affinity interaction.

Schluep T, Cooney CL.

Chemical Engineering Department, Massachusetts Institute of Technology, 7 Massachusetts Avenue, Cambridge, MA 02139, USA.
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Production of pharmaceutical grade plasmid DNA is an important issue in gene therapy. We developed a method for affinity purification of plasmids by triple helix interaction. This method is based on sequence-specific binding of an oligonucleotide immobilized on a large pore chromatography support to a target sequence on the plasmid. Using design criteria derived from thermodynamic data, we produced a 15mer target sequence which binds strongly to the affinity support under mildly acidic conditions. Plasmid DNA was purified from clarified Escherichia coli lysate by incubation with the affinity beads at pH 5.0 and high NaCl concentration. After extensive washir of the beads, purified plasmid DNA was eluted with alkaline buffer. The purified plasmid showed no RNA or cell DNA contamination in HPLC analysis and total protein concentration was reduced considerably. Due to its mechanical stability and porosity this support can be used in a continuous affinity purification process, which has a high potential for scale up.

PMID: 9742258 [PubMed - indexed for MEDLINE]

1: J Chromatogr A 1998 Aug 7;816(1):107-11 Related Articles, Boo

Recent developments of magnetic beads for use in nucleic acid purification.

Levison PR, Badger SE, Dennis J, Hathi P, Davies MJ, Bruce IJ, Schimkat D.

Whatman International Ltd., Maidstone, Kent, UK.

The performance of Magarose, an agarose-based bead containing a paramagnetic component has been evaluated. The anion exchanger DEAE-Magarose is effective at binding DNA from a crude cell lysate. The plasmid pBluescript was isolated from 1.5 ml Escherichia coli JM109 cell culture, following alkaline lysis yielding 8.2 micrograms high-quality DNA. Under similar binding conditions 21 micrograms of salmon sperm DNA bound to th ion exchangers. The affinity medium oligo-dT Magarose was demonstrated t bind 75 mumol of an oligo-dA probe/g of medium by hybridization. Under similar conditions mRNA could be isolated from a preparation of baby hamster cell total RNA. The magnetic susceptibility of Magarose is very higl facilitating the use of this separation technique for rapid batch chromatographic processes.

PMID: 9741104 [PubMed - indexed for MEDLINE]

1: Nucleic Acids Res 1997 Apr 15;25(8):1660-1

Large scale preparation of positively supercoiled DNA using the archaeal histone HMf.

LaMarr WA, Sandman KM, Reeve JN, Dedon PC.

Division of Toxicology, 56-787, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, USA.

A technique to prepare relatively large quantities (>/=100 microg) of highly positively supercoiled DNA is reported. This uses a recombinant archaeal histone (rHMfB) to introduce toroidal supercoils, and an inexpensive chicker blood extract to relax unrestrained superhelical tension. Preparation of positively supercoiled pUC19 DNA molecules, >50% of which have linking number changes ranging from+8 to+17, is demonstrated. Advantages include the high degree of positive supercoiling that can be achieved, control over th extent of supercoiling, easy production of relatively large quantities of supercoiled DNA, and low cost.

PMID: 9092677 [PubMed - indexed for MEDLINE]

1: Nucleic Acids Res 1997 Mar 15;25(6):1315-6

Simple and rapid preparation of plasmid template by a filtratio method using microtiter filter plates.

Itoh M, Carninci P, Nagaoka S, Sasaki N, Okazaki Y, Ohsumi T, Muramatsu M, Hayashizaki Y.

Genome Science Laboratory, Tsukuba Life Science Center, The Institute of Physical and Chemical Research (RIKEN), Koyadai 3-1-1, Tsukuba-city, Ibaraki 305, Japan.

We developed a new simple high-throughput plasmid DNA extraction procedure, based on a modified alkaline lysis method, using only one 96-wel microtiter glassfilter plate. In this method, cell harvesting, lysis by alkaline and plasmid purification are performed on only one microtiter glassfilter plate. After washing out RNAs or other contaminants, plasmid DNA is elute by low-ion strength solution, although precipitated chromosomal DNA is not eluted. The plasmid prepared by this method can be applied to sequencing reactions or restriction enzyme cleavage.

PMID: 9092649 [PubMed - indexed for MEDLINE]

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1: Biotechniques 1998 Feb;24(2):240-3 Related Articles, Boo

Rapid resuspension of pelleted bacterial cells for miniprep plasmid DNA isolation.

Voo KS, Jacobsen BM.

Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis 46202-5225, USA.

PMID: 9494723 [PubMed - indexed for MEDLINE]

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1: J Biotechnol 2001 Feb 23;85(3):297-304

Purification of essentially RNA free plasmid DNA using a modified Escherichia coli host strain expressing ribonuclease A.

Cooke GD, Cranenburgh RM, Hanak JA, Dunnill P, Thatcher DR, War JM.

The Advanced Centre For Biochemical Engineering, Department of Biochemical Engineering, University College London, Torrington Place, WC1E 7JE, London, UK

Regulatory agencies have stringent requirements for the large-scale production of biotherapeutics. One of the difficulties associated with the manufacture of plasmid DNA for gene therapy is the removal of the host cell related impurity RNA following cell lysis. We have constructed a modified Escherichia coli JM107 plasmid host (JMRNaseA), containing a bovine pancreatic ribonuclease (RNaseA) expression cassette, integrated into the ho: chromosome at the dif locus. The expressed RNaseA is translocated to the periplasm of the cell, and is released during primary plasmid extraction by alkaline lysis. The RNaseA protein is stable throughout incubation at high pH (approximately 12-12.5), and subsequently acts to hydrolyse host cell RNA present in the neutralised solution following alkaline lysis. Results with this strain harbouring pUC18, and a 2.4 kb pUC18DeltalacO, show that sufficien levels of ribonuclease (RNase) activity are produced to hydrolyse the bulk of the host RNA. This provides a suitable methodology for the removal of RNA whilst avoiding the addition of exogenous animal sourced RNase and its associated regulatory requirements.

PMID: 11173096 [PubMed - in process]

1: J Biotechnol 2000 Jan 21;76(2-3):175-83

Effects of growth medium selection on plasmid DNA production and initial processing steps.

O'Kennedy RD, Baldwin C, Keshavarz-Moore E.

The Advanced Centre for Biochemical Engineering, Department of Biochemical Engineering, University College London, UK.

Cultures of recombinant Escherichia coli containing the plasmid pSVbeta were grown in three medium formulations to assess their effects on the characteristics of supercoiled plasmid DNA production for plasmid-based gene therapy. A semi-defined medium containing casamino acids (SDCAS) was found to support higher cell densities and higher plasmid stability than a similar medium containing soya amino acids (SDSOY) or Luria-Bertani medium (LB). Differences were observed in the cell harvest characteristics, plasmid DNA primary recovery, plasmid DNA yield and quality between cells grown on LB and on SDCAS medium. Cells grown on SDCAS medium were more difficult to resuspend after harvest than those grown in LB medium and were less susceptible to alkaline lysis. The plasmid DNA conter from SDCAS was predominantly supercoiled and was less contaminated by chromosomal DNA than plasmid DNA extracts derived from cells grown on LB medium. It was hypothesised that the different carbon:nitrogen ratio (C:N of the medium may have been responsible for changing the cell wall polysaccharide composition resulting in the change in cell harvest and lysis characteristics. Results indicated that changing the C:N ratio of SDCAS medium between 1.21:1 and 12.08:1 resulted in no alteration in cell wall polysaccharide composition or in cell susceptibility to chemical lysis or physical breakage. Plasmid DNA yields increased ten-fold with ten-fold increase in the C:N ratio of SDCAS medium.

PMID: 10656332 [PubMed - indexed for MEDLINE]

1: Biotechniques 1999 Mar;26(3):518-22, 524, 526 Related Articles, Boo

Fast and accurate method for quantitating E. coli host-cell DNA contamination in plasmid DNA preparations.

Smith GJ 3rd, Helf M, Nesbet C, Betita HA, Meek J, Ferre F.

Althea Technologies, San Diego, CA, USA.

Plasmid DNA is being used successfully as a gene delivery vector in a variet of clinical applications. Similar to other pharmaceutical products for clinical use, the plasmid vectors must meet rigorous purity standards. One important contaminant is the DNA of the host cell used to produce the plasmids. We have developed a new method to accurately quantitate E. coli host-cell DNA in plasmid preparations. This method is based on kinetic PCR using the ABI PRISM 7700 with 23S rDNA as a target. This precise assay is significantly faster and has a lower limit of quantitation than the currently used Southern-based methods.

PMID: 10090994 [PubMed - indexed for MEDLINE]

1: Biotechnol Bioeng 2000 Jun 5;68(5):576-83

Purification of a cystic fibrosis plasmid vector for gene therapy using hydrophobic interaction chromatography.

Diogo MM, Queiroz JA, Monteiro GA, Martins SA, Ferreira GN, Prazeres DM.

Centro de Engenharia Biologica e Quimica, Instituto Superior Tecnico, Av. Rovisco Pais, 1000 Lisboa, Portugal.

The success and validity of gene therapy and DNA vaccination in in vivo experiments and human clinical trials depend on the ability to produce large amounts of plasmid DNA according to defined specifications. A new methoc is described for the purification of a cystic fibrosis plasmid vector (pCF1-CFTR) of clinical grade, which includes an ammonium sulfate precipitation followed by hydrophobic interaction chromatography (HIC) using a Sepharose gel derivatized with 1,4-butanediol-diglycidylether. The use of HIC took advantage of the more hydrophobic character of single-stranded nucleic acid impurities as compared with double-stranded plasmid DNA. RNA, denatured genomic and plasmid DNAs, with large stretches of single strands, and lipopolysaccharides (LPS) that are more hydrophobic than supercoiled plasmid, were retained and separated from nonbinding plasmid DNA in a 14-cm HIC column. Anion-exchange HPLC analysis proved that >70% of the loaded plasmid was recovered after HIC. RNA and denatured plasmid in the final plasmid preparation were undetectable by agarose electrophoresis. Other impurities, such as host genomic DNA and LPS, were reduced to residual values with the HIC column (<6 ng/&mgr;g pDNA and 0.048 EU/&mgr;g pDNA, respectively). The total reduction in LPS load in the combined ammonium acetate precipitation and HIC was 400,000-fold. Host proteins were not detected in the final preparation by bicinchoninic acic (BCA) assay and sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with silver staining. Plasmid identity was confirmed by restriction analysis and biological activity by transformation experiments. Th process presented constitutes an advance over existing methodologies, is scaleable, and meets quality standards because it does not require the use of additives that usually pose a challenge to validation and raise regulatory concerns. Copyright 2000 John Wiley & Sons, Inc.

1: Protein Expr Purif 2000 Oct;20(1):21-6

The delta-endotoxin proteins accumulate in Escherichia coli as a protein-DNA complex that can be dissociated by hydrophobic interaction chromatography.

Chaturvedi R, Bhakuni V, Tuli R.

Molecular Biology and Genetic Engineering Division, National Botanical Research Institute, Rana Pratap Marg, Lucknow, 226001, India.

The insecticidal protein CryIAc accumulated to form inclusion bodies in Escherichia coli upon overexpression of the cloned gene. The solubilized inclusion bodies contained the delta-endotoxin in association with DNA fragments of about 25 kb. The protein-DNA complex could be dissociated and the delta-endotoxin purified by hydrophobic interaction chromatography on phenyl-Sepharose. The DNA was washed out in the high-salt buffer while the delta-endotoxin was bound to the matrix and was eluted at 4 degrees C by a stepwise decreasing potassium chloride gradient. The DNA-protein comple also contained plasmids harbored by the host strain. The plasmid DNA associated with the complex became competent to transform E. coli only afte it was dissociated from the delta-endotoxin. The hydrophobic interaction chromatography provides an efficient method for the purification of DNA-free activated toxin. Copyright 2000 Academic Press.

PMID: 11035946 [PubMed - indexed for MEDLINE]

1: Mol Biotechnol 2001 Jan;17(1):59-64

Rapid extraction and purification of environmental DNA for molecular cloning applications and molecular diversity studies.

Santosa DA.

Department of Soil Science, Faculty of Agriculture, Bogor Agricultural University, and Indonesian Center for Biodiversity and Biotechnology (ICBB), R.E. Martadinata 8, Bogor 16162, Indonesia. dsantosa@indo.net.id

A rapid method for the extraction and purification of DNA from environmental samples for molecular cloning applications was developed. The indigenous cells from plant debris, organic materials, sediments, and soi were lysed directly by using DAS-IZ solution and the nucleic acids were precipitated with isopropanol. A simple purification step using DAS-IIZ solution without binding matrix produced highly pure, colorless and undegraded DNA with molecular weight of more than 20 kb. The superiority of this method was tested for wide applications in molecular cloning, i.e., construction of genomic library by using Lambda DASHII Vector and GigapackIII XL, plasmid library, cloning of gene encoding protease, and molecular microbial diversity analysis. An additional advantage of this method is that only 0.1 g of sample is required, if analysis of many samples i short time should be done. To extract large amounts of environmental DNA for molecular cloning lasts only 30 min and to purify it less than 1 h.

PMID: 11280931 [PubMed - in process]

1: Nucleic Acids Res 2000 Aug 15;28(16):E76

Efficient purification of DNA fragments using a protein binding membrane.

Ihle O, Michaelsen TE.

National Institute of Public Health, Department of VAIM, PO Box 4404 Torshov, 0462 Oslo, Norway. oistein.ihle@folkehelsa.no

A novel and efficient method has been developed for isolation of correctly digested DNA fragments without the use of classic size-dependent electrophoretic separation methods. To achieve this, DNA fragments are end labelled by haptens. After specific endonuclease digestion of the hapten-labelled DNA, the DNA is incubated with a protein that specifically binds to the hapten. The incubation mixture is then passed through a cartridge containing a protein-binding membrane that does not bind DNA. Undigested and partly digested DNA are retained on the membrane, while correctly digested DNA is selectively recovered for use in further downstream applications.

PMID: 10931935 [PubMed - indexed for MEDLINE]

1: Biotechnol Bioeng 2000 Jul 5;69(1):101-6

Purification of plasmid DNA by tangential flow filtration.

Kahn DW, Butler MD, Cohen DL, Gordon M, Kahn JW, Winkler ME.

Department of Recovery Science, Genentech, Inc., 1 DNA Way, South San Francisco, California, USA. kahn.david@gene.com

A simple, scalable method for purification of plasmid DNA is described. The method includes modification of the classical alkaline-lysis-based plasmid extraction method by extending the solubilization step from less than 30 min to 24 h. The extraction is followed by the novel use of tangential flow filtration (TFF) for purification of the remaining contaminants. The method does not include the use of any organic solvents, RNase, high-speed centrifugation, or column chromatography steps. The method typically yields 15 to 20 mg of plasmid DNA per liter of bacterial culture and results in removal of >99% of RNA and >95% of the protein that remains after the modified alkaline lysis procedure. The procedure has been demonstrated to b effective in the isolation of seven different plasmids. Plasmids isolated using this method had comparable transfection capability relative to plasmid isolated using a classical, cesium chloride gradient-based method. Copyright 2000 John Wiley & Sons, Inc.

PMID: 10820336 [PubMed - indexed for MEDLINE]

1: Lett Appl Microbiol 2000 Jan;30(1):38-41

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Development of simple and efficient protocol for isolation of plasmids from mycobacteria using zirconia beads.

Madiraju MV, Qin MH, Rajagopalan M.

Department of Biochemistry, The University of Texas Health Center at Tyle 75710, USA.

A two-step protocol has been developed for isolation of plasmids from recombinant mycobacteria via Escherichia coli. First either mycobacterial primary transformants or propagated cultures were lysed in a mini-bead beat using zirconia beads and the lysate thus obtained was used to transform E. coli recA mutant cells. Secondly, plasmid DNA was isolated from recombinant E. coli cells and analysed. Bead beating times of 2 min for Mycobacterium smegmatis, a rapid grower, and 4 min for M. bovis BCG, a slow grower, were found to be optimal for recovery of plasmid DNA. This protocol was also amenable to other mycobacterial species such as M. avium M. fortuitum and M. tuberculosis H37Ra. Plasmid recovery from the recombinant M. bovis BCG using this protocol is approximately 300-fold higher than that reported for the electroduction method.

PMID: 10728558 [PubMed - indexed for MEDLINE]

1: J Biotechnol 2000 Jan 21;76(2-3):197-205 Related Articles, Boo

Removal of contaminant nucleic acids by nitrocellulose filtratio during pharmaceutical-grade plasmid DNA processing.

Levy MS, Collins IJ, Tsai JT, Shamlou PA, Ward JM, Dunnill P.

The Advanced Centre for Biochemical Engineering, Department of Biochemical Engineering, University College London, UK.
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Pharmaceutical-grade plasmid DNA for use in vaccines and gene therapy requires the development of reproducible and scaleable downstream processes. Shearing of chromosomal DNA at the commencement of the purification results in fragments that are difficult to separate from supercoile plasmid DNA. Regulatory standards will probably require that the level of chromosomal DNA contamination is kept below 0.01 mg mg(-1) plasmid DNA. This work reports the use of nitrocellulose membranes to decrease chromosomal DNA contamination in plasmid DNA preparations derived fro a 450-l bioreactor. Clarified lysates, resuspended PEG precipitates and anion exchange chromatography elutes were filtered through nitrocellulose. In all the cases, chromosomal DNA was selectively retained by the membrane whi most supercoiled plasmid DNA was recovered in the filtrate. Contamination levels dropped from over 27% to below 1% as measured by Southern analysis. Under ionic strength conditions equal to or above 1.5 M NaCl, a fraction of the contaminant RNA was also retained by the nitrocellulose membrane.

PMID: 10656334 [PubMed - indexed for MEDLINE]

1: Int Microbiol 1999 Jun;2(2):115-7 Related Articles, Boo

A rapid procedure for the isolation of plasmid DNA from environmental bacteria.

Ferrus MA, Alonso JL, Amoros I, Hernandez M, Hernandez J.

Department of Biotechnology, Polytechnic University of Valencia, Spain. mferrus@btc.upv.es

The INSTA-MINI-PREP method, a rapid protocol for plasmid DNA extraction, was originally developed to prepare plasmid DNA from 1 to 3 ml miniprep Escherichia coli cultures. Direct extraction of plasmid DNA is achieved by a two-phase solution which is separated by centrifugation in the presence of the INSTA-PREP gel barrier material. This method has been successfully tested on various environmental Salmonella strains, although it was not suitable for Pseudomonas aeruginosa and enterococci strains. The INSTA-MINI-PREP method is a new alternative procedure to screen plasmic contents of Salmonella and E. coli strains rapidly and easily.

PMID: 10943402 [PubMed - indexed for MEDLINE]

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